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Helix–Coil Stability Constants for the Naturally Occurring Amino Acids in Water. 15. Arginine Parameters from Random Poly(hydroxybutylglutamine-*co*-L-arginine)[1]

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Received July 8, 1977

ABSTRACT: Water-soluble copolymers containing L-arginine and N^5-(4-hydroxybutyl)-L-glutamine were prepared by copolymerization of the N-carboxy-α-amino acid anhydrides of N^δ-*tert*-butyloxycarbonyl-L-ornithine and γ-benzyl L-glutamate, followed by aminolysis with 4-amino-1-butanol, by removal of the *tert*-butyloxycarbonyl protecting group, and by treatment with O-methylisourea. The copolymers were fractionated and characterized, and the thermally induced helix–coil transitions of these copolymers were studied in water at neutral pH in the presence and in the absence of KCl. The Zimm–Bragg parameters σ and s for the helix–coil transition in poly(L-arginine) in aqueous solution were deduced from an analysis of the melting curves of the copolymers in the manner described in earlier papers. The computed values of s indicate that L-arginine is a weak helix-making residue at low temperature and a weak helix-breaking residue at high temperature in aqueous solution. The results were found to be in good agreement with those obtained earlier in conformational analyses of arginyl residues in proteins.

The use of the "host–guest" technique for the evaluation of the helix–coil stability constants of various amino acids in water has been illustrated in earlier papers of this series, the latest of which was paper 14.[2] In the present paper, this approach is extended to L-arginine. In the "host–guest" technique, a water-soluble, α-helical host homopolymer with nonionizable side chains is selected, and various amounts of a guest residue are incorporated into it to form random copolymers. The use of random copolymers, in which L-arginine is the minor component, allows the measurement of the helix–coil transition properties of charged arginine residues at neutral pH in the absence of long-range electrostatic interactions between arginine residues.[3] Thus, it is possible[2,3] to determine the Zimm–Bragg[4] parameters σ and s for the guest residues by examining their influence on the helix–coil transition properties of the host homopolymers. L-Arginine residues are incorporated into a copolymer with an N^5-(4-hydroxybutyl)-L-glutamine host, and the thermally induced helix–coil transition in these copolymers in water at neutral pH is examined.

The synthesis of water-soluble random copolymers of L-arginine with N^5-(4-hydroxybutyl)-L-glutamine is described in section I, and the experimental characterization of these copolymers and their melting behavior in aqueous solution are presented in section II. Finally, in section III, the data are analyzed by means of an appropriate form of the theory[5] to determine the helix–coil stability parameters of L-arginine in water. These are compared with empirical observations on the behavior of this residue in proteins.

I. Experimental Section. Preparation and Characterization of the Copolymers

The synthesis of the copolymers was achieved by first copolymerizing the N-carboxyanhydrides (NCA's) of N^δ-*tert*-butyloxycarbonyl-L-ornithine and γ-benzyl L-glutamate in dioxane using sodium methoxide as an initiator. The resulting copolymers were then converted to the 4-hydroxybutylglutamine derivatives by treatment with 4-amino-1-butanol, and, afterwards, the δ-amino protecting group of L-ornithine was removed using 3 N HCl. The water-soluble copolymers were then treated with O-methylisourea in water at pH 10.0 at 0–4 °C to give copolymers of N^5-(4-hydroxybutyl)-L-glutamine with L-arginine [see Katchalski and Spitnik[6] for the preparation of poly(L-arginine) from poly(L-ornithine)].

A. Materials. The solvents and reagents used for the preparation and purification of the starting amino acid derivatives and the NCA's were purified shortly before use (see previous papers in this series[2,3]). Acetonitrile was dried over molecular sieves (4 Å) for 2 days and then refluxed and distilled over calcium hydride. L-Ornithine was purchased from Mann Research Labs., and *tert*-butyloxycarbonyl azide and O-methylisourea hydrogen sulfate were purchased from Aldrich Chem. Co.

B. Synthesis. N-Carboxyanhydrides. N^δ-*tert*-Butyloxycarbonyl-L-ornithine was prepared from the copper complex of L-ornithine and *tert*-butyloxycarbonyl azide, followed by treatment of the isolated Cu complex with hydrogen sulfide, according to Tesser and Schwyzer.[7]

To a suspension of N^δ-*tert*-butyloxycarbonyl-L-ornithine and AgCN in freshly distilled acetonitrile, a solution of phosgene in di-

Table I
Compositions and Chain Lengths of Unfractionated Poly[Glu(OBzl),Orn(Boc)]

Polymer No.	L-Orn content of reaction mixture, mol %	L-Orn content found,[a] mol %	Av mol wt[b] $\times 10^{-3}$	$\overline{\mathrm{DP}}$
I	5	4.99	219	1109
II	10	11.0	270	1230
III	12	11.9	280	1283
IV	18	17.7	210	962

[a] In the amino acid analysis, the measured color value for Orn relative to that of Gly (1.0) was found to be 0.65. [b] By viscometry using the relationship of Fujita et al.[11] for polymers in dichloroacetic acid.

oxane was added, as in the procedure of Hirschmann et al.[8] for the synthesis of the NCA of N^{ϵ}-*tert*-butyloxycarbonyl-L-lysine. Two recrystallizations from ethyl acetate and hexanes gave a 49% yield of colorless material: mp 138.5–139 °C; $[\alpha]_D{}^{21}$ −20.0° (*c* 0.71, acetone). Anal. Calcd for $C_{11}H_{18}N_2O_5$: C, 51.16; H, 7.03; N, 10.85. Found: C, 51.2; H, 7.2; N, 10.8.

γ-Benzyl L-glutamate *N*-carboxyanhydride was prepared by the general method described by Hirschmann et al.[8]

Poly[γ-benzyl L-glutamate-*co*-N^{δ}-*tert*-butyloxycarbonyl-L-ornithine], Poly[Glu(OBzl), Orn(Boc)], Copolymers I–IV. Random copolymers of γ-benzyl L-glutamate and 5 to 18 mol % of N^{δ}-*tert*-butyloxycarbonyl-L-ornithine were prepared by polymerizing mixtures of the two *N*-carboxyanhydrides in dioxane with sodium methoxide as initiator, using an A/I ratio of 40. The progress of the polymerization reactions was monitored as described in paper 10 of this series,[9] and the polymers were isolated after completion of the reaction as usual.[10] The chain lengths of these polymers, determined roughly with the viscosity–molecular weight relationship of Fujita et al.,[11] are given in Table I.

Poly[N^5-(4-hydroxybutyl)-L-glutamine-*co*-L-arginine], Poly(HBG,Arg), Copolymers V–VIII. The copolymers I–IV were treated with 4-amino-1-butanol to convert them into the corresponding water-soluble copolymers, as described previously.[12] The course of the aminolysis was monitored as described in paper 10[9]. The reaction was terminated by pouring the cooled mixture slowly into cold aqueous HCl, sufficiently concentrated to give ca. 2 N HCl following the neutralization of excess 4-amino-1-butanol in the reaction mixture. After 20 min, enough cold, concentrated HCl was added to give a final concentration of ca. 3 N. Stirring at 0–4 °C was continued for another 20 min and then at room temperature for ca. 1 h. The solution was diluted and dialyzed against water until the dialyzate exhibited a negative ninhydrin test.[13]

After lyophilization, ca. 1.0 g of the copolymer was dissolved in 40 mL of H_2O, and the solution was cooled in an ice bath. A cold solution of 8.43 g of *O*-methylisourea hydrogen sulfate in 20 mL of H_2O (whose pH had been adjusted to 10.0 with 4 N NaOH) was added in aliquots, with stirring.[14] When the reaction mixture turned cloudy, a few milliliters of cold water was added until it cleared again. Then, over a period of 6 days, additional amounts of *O*-methylisourea hydrogen sulfate (4.24 g in 20 mL of water, with the pH adjusted to 10.0) were added in aliquots to the reaction mixture: two additions in the case of the 5% Orn copolymer, three additions for the 10 and 12% copolymers, and four additions for the 18% copolymer reaction mixture. The conversion of ornithine to arginine was checked after 7–8 days by removing a small sample from the reaction mixture, desalting it on Sephadex G-10 after acidification, and determining the amino acid composition after hydrolysis in 12 N HCl for 24 h at 105 °C. The 5, 10, and 12% Orn copolymers were converted completely into the corresponding Arg copolymers (no or negligible amounts of Orn could be detected on the analyzer). The reaction mixture was then acidified, dialyzed extensively against H_2O, and finally lyophilized. Yields ranged from 820 to 850 mg (78–81% yields).

The 18% Orn copolymer, however, behaved differently; the amidination reaction mixture remained turbid even after dilution with H_2O and remained that way (before the addition of the *O*-methylisourea solution, the polymer solution had been clear). When four additions of the reagent had been added, this turbid solution was dialyzed against dilute HCl (after 10 days of reaction time) and a precipitate formed in the dialysis bag. After stirring overnight, the precipitate dissolved, and the slightly turbid solution became clear after dialysis against water for 3 more days. Amino acid analysis showed that only about 5–6% of the original 18% ornithine had been converted (after the exchange reaction). When the lyophilized material was treated again with *O*-methylisourea in the cold (at pH 10.0), only about 2% of the remaining ornithine was converted after 7 days. When the reaction was carried out in 8 M urea (initial concentration) the conversion of ornithine into arginine could not be improved. To check if this behavior was caused by incomplete deprotection of the Boc groups, and thus incomplete conversion to arginine, about 400 mg of the material that had been subjected to the amidination procedure twice was dissolved in 40 mL of 3 N HCl, and the solution was kept at room temperature for ca. 1.5 h. It was then dialyzed against water until the dialyzate was neutral, and then a cold solution of 8.43 g of *O*-methylisourea hydrogen sulfate in 20 mL of H_2O (pH adjusted to 10) was added to the polymer solution (ca. 80 mL). Two more portions of *O*-methylisourea hydrogen sulfate (each of 4.24 g in 15 mL of H_2O) were added and, after 11 days, the reaction mixture was worked up as described above. Amino acid analysis showed that Orn in the copolymer was completely converted into Arg. This indicates that, even though deprotection of Orn is difficult when the Orn content is high, nevertheless it can be accomplished.

C. Fractionation. Unlike the copolymers studied previously,[2,3,9,10,12,15] poly[HBG,Arg] was not very soluble in methanol. Therefore, the copolymers were dissolved in a mixture of methanol and 1 M aqueous NaCl (9:1, by volume) for fractionation. (The salt was added to reduce electrostatic effects arising from the ionized side chain of the arginine residues, so that the fractionation would be based on molecular weight.) Ether was added, and several fractions were isolated as usual.[15] After isolation of the fractions, the residues were dissolved in H_2O, dialyzed against H_2O until the dialyzate was negative to chloride, and finally lyophilized.

D. Analytical Methods. Determination of Composition. The copolymer fractions were hydrolyzed in 6 or 12 N HCl at 105 °C for 24 h in evacuated, degassed, sealed ampules and were worked up according to the procedure of Spitz[16] in order to prevent artifact formation.[17] A Technicon TSM amino acid analyzer was used. The average experimental error in determination of the composition was ±6.6%.

Determination of Concentration. The concentrations of all copolymer solutions were determined by micro Kjeldahl nitrogen analysis according to Lang[18] or by a combination of Lang's method for digestion[18] and the semiautomated method of detection of ammonia of Noel and Hambleton.[19]

Optical Purity. The monomeric starting material as well as the acid hydrolyzates of the intermediate and final copolymers were assayed for the presence of D isomers using the dipeptide method of Manning and Moore.[20] The hydrolyzate mixture was separated into two fractions, one of which contained glutamic acid and the other ornithine and 4-amino-1-chlorobutane (to which 4-amino-1-butanol was converted during hydrolysis in 12 N HCl for 24 h), or arginine and 4-amino-1-chlorobutane, prior to derivatization. The separation was achieved by means of an ion-exchange column (60 mm × 5 mm, contained in a disposable pipet) of Dowex AG1-X8 (200–400 mesh) in the hydroxyl form. The samples were eluted with water followed by 0.5 N acetic acid.

The glutamic acid from this separation was coupled with L-Leu-NCA and the resulting diastereoisomeric dipeptides L-Leu-L-Glu and L-Leu-D-Glu were separated and quantitatively determined on a Technicon amino acid analyzer using a sodium citrate elution buffer of pH 3.9 (0.2 N NaCl). The mixture of ornithine and 4-amino-1-chlorobutane was reacted with L-Glu-NCA, as with lysine,[3,20] and the resulting peptides $N^{\alpha,\delta}$-(di-L-Glu)-D-Orn, $N^{\alpha,\delta}$-(di-L-Glu)-L-Orn, and glutamic acid-α-(1-chlorobutyl)-amide were separated and determined quantitatively on a Technicon amino acid analyzer using a sodium citrate elution buffer of pH 4.3 (0.2 N NaCl). The mixture of arginine and 4-amino-1-chlorobutane was reacted with L-Glu-NCA,[3,20] and the resulting peptides L-Glu-L-Arg, L-Glu-D-Arg, and glutamic acid-α-(1-chlorobutyl)-amide were separated and determined quantitatively on a Technicon amino acid analyzer using a sodium citrate elution buffer of pH 5.4 (0.2 N NaCl). Appropriate peptide standards were prepared from D,L-ornithine, D,L-arginine, and D,L-glutamic acid.

E. Viscosity, ORD, and CD Measurements. Viscosity, optical rotatory dispersion (ORD), and circular dichroism (CD) measurements were all carried out as described previously.[15]

F. Molecular Weights. The molecular weight of fractions from samples V–VIII were determined by the conventional sedimentation equilibrium method as reported earlier,[15] with the following exception. The polymers were dissolved in 0.2 M KCl solution and dialyzed against a large volume of 0.2 M KCl solution overnight, in order to eliminate polyelectrolyte effects.[21] The dialyzate was used as the

Table II
Characterization of the Fractionated Copolymers

Fraction[a]	L-Arg content, mol %	$\overline{M}_w \times 10^{-3}$ [b]	$\overline{M}_z/\overline{M}_w$	$\overline{DP}_w$
VB	4.3	205.2	1.50	1036
VDA[c]	4.2	70.3	1.32	367
VIIB	10.4	125.9	1.20	648
VIIE	10.3	45.8	1.24	236
VIIIC	16.0	125.3	1.04	650

[a] Samples V, VII, and VIII are derived from the unfractionated copolymers I, III, and IV, respectively. The letters correspond to the fractions obtained in the fractionation procedure. [b] This value was obtained by conventional sedimentation equilibrium (with extrapolation to zero concentration). [c] Fraction VDA was derived from a refractionation of fraction VD.

solvent in the molecular weight measurements. The concentration dependence of the weight-average molecular weight, $\overline{M}_w$, was determined for each sample, and $\overline{M}_z$ was computed from the run at the lowest concentration for each fraction. The estimated precision in the values of $\overline{DP}_w$ was ±5%.

The partial specific volumes ($\bar{v}$) of the several fractions, required for the calculation of molecular weights, were determined from the amino acid content as described by Cohn and Edsall.[22] A value of $\bar{v}$ = 0.816 for PHBG was used in the calculation of $\bar{v}$ for the copolymers.[15]

II. Results

A. Characterization of the Copolymers. Table I summarizes the composition and the average degree of polymerization ($\overline{DP}$) of the unfractionated poly[Glu(OBzl),Orn(Boc)] copolymers. Table II summarizes the molecular weight and composition data for the corresponding fractionated copolymers poly(HBG,Arg). The usual decrease in $\overline{DP}_w$ attributed to transaminolysis upon conversion to the hydroxybutylglutamine polymers is apparent from a comparison of the two tables. However, the poly[Gly(OBzl),Orn(Boc)] and the corresponding poly(HBG,Arg) copolymers had similar compositions of guest residues. Also the composition is essentially independent of chain length for a given parent copolymer, i.e., the same average amount of arginine is found in the short chains as in the long chains within experimental error. These observations indicate that there is little departure from randomness in the amino acid sequence in these copolymers. Furthermore, it has been demonstrated in paper I[5] and discussed in paper XI[3] of this series that the presence of fairly large size blocks (i.e., small deviations from randomness) does not influence the melting behavior of these copolymers. While this criterion has been used in all previous papers in this series to indicate that the polymers were sufficiently random for the purpose required here, a more rigorous proof was provided for copolymers containing methionine as the guest residue, by analyzing the distribution of fragments from a CNBr digest of the copolymer.[23]

The concentration dependences of the apparent molecular weights of the fractions studied, illustrated in Figure 1, are small in most cases, and the data have been extrapolated to infinite dilution to obtain $\overline{DP}_w$. The values of $\overline{M}_z/\overline{M}_w$ for most fractions do not depart significantly from unity, indicating that the fractions used in the determination of σ and s were relatively homogeneous. Fraction VB had a high value of $\overline{M}_z/\overline{M}_w$; however, the melting behavior is not sensitive to the heterogeneity in such a highly polymerized polypeptide.[5] Therefore, even copolymer fraction VB could be used in the analysis.

Poly(HBG,Orn) and unfractionated poly(HBG,Arg), which were derived from polymer No. III, were hydrolyzed and analyzed for optical purity using the method of Manning and Moore.[20] No racemization was found for ornithine, arginine, or hydroxybutylglutamine residues (within 1.7%); all samples were, therefore, considered to be optically pure enough for use in the computation of the values of σ and s for L-arginine.

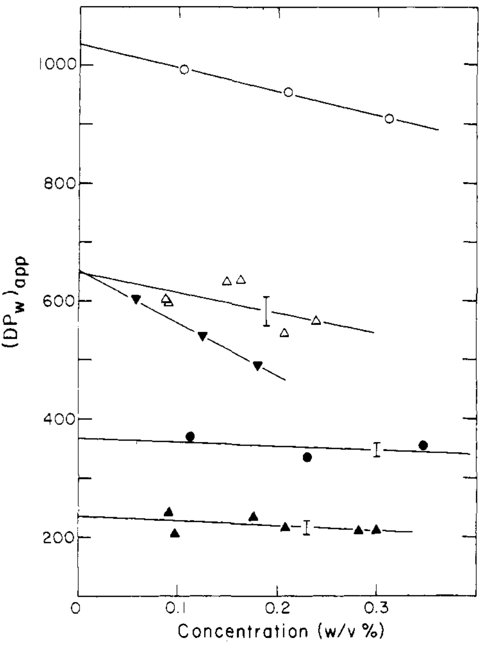

Figure 1. Concentration dependence of molecular weights for fractions used in analysis to obtain σ and s: (○) 4.3% Arg, $\overline{DP}_w$ = 1036 (fraction VB); (●) 4.2% Arg, $\overline{DP}_w$ = 367 (fraction VDA); (△) 10.4% Arg, $\overline{DP}_w$ = 648 (fraction VIIB); (▲) 10.3% Arg, $\overline{DP}_w$ = 236 (fraction VIIE); (▼) 10.6% Arg, $\overline{DP}_w$ = 650 (fraction VIIIC).

B. b_0 for Complete Helix and Complete Coil. For the homopolymer host poly(HBG) studied in paper 2,[15] the value of b_0 corresponding to the complete helix was assigned as −750 and that corresponding to the complete coil was taken as zero. Because the value may vary with the nature of the side chain,[24] it was necessary to determine the respective values of b_0 for the copolymers with arginine. The value of b_0 for copolymer fraction VIIB (10.4% Arg, $\overline{DP}_w$ = 648) was measured in 90% aqueous methanol at 1 °C. After correcting the data for the dispersion of the refractive index of the solvent using the Sellmeier equation,[25] a b_0 value of −720 was obtained. This result indicates that the value of −750 is a reasonable one for the complete helix in these copolymers. Similarly, the value of 24 was obtained for fraction VIIB in DCA at 25 °C, and it indicates that zero is a reasonable one for the complete coil. Thus, the helix content, θ_h, was computed as $-b_0/750$.

C. ORD and CD Data. The ORD and CD spectra for representative fractions of poly(HBG,Arg) in water at neutral pH are shown in Figure 2. Both the ORD and the CD spectra indicate the presence of a right-handed α-helical structure[26-28] mixed with random coil. Contributions to these spectra arising from β structure are not evident. With increasing temperature, the ORD spectra (not shown here) become characteristic of the random coil mixed with small amounts of α helix, indicating that these copolymers undergo a thermally induced transition from α helix to random coil in water. The thermally induced helix–coil transition curves were obtained by measuring the temperature dependence of b_0 and taking θ_h (fraction helix) to be $-b_0/750$. Transition curves were obtained for the five fully characterized fractions (listed in Table II) in water at neutral pH in the presence and in the absence of KCl. The helix content of fraction VIIIC with the highest arginine content (16.0% Arg) was influenced by added salt as observed for poly(HBG,Lys) copolymers of high lysine content (19.5% Lys).[3] Figure 3 shows how the helix content increases

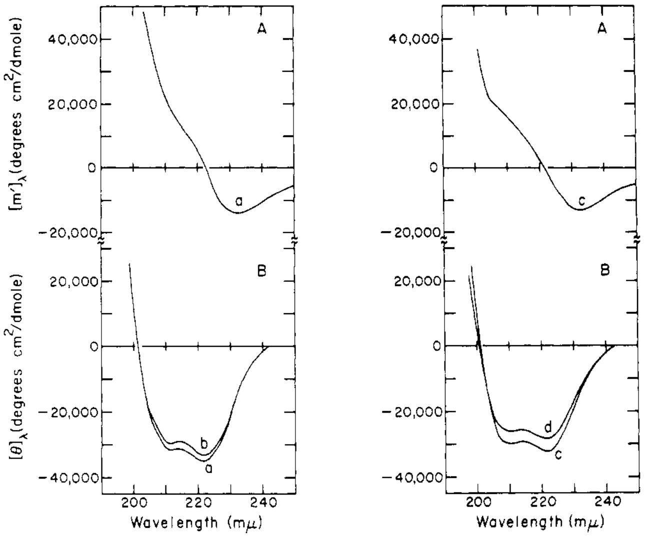

Figure 2. (A) ORD and (B) CD data in water at 25 °C and at neutral pH for four poly(HBG,Arg) copolymers: (a) 4.3% Arg, $\overline{DP}_w$ = 1036 (fraction VB); (b) 4.2% Arg, $\overline{DP}_w$ = 367 (fraction VDA); (c) 10.4% Arg, $\overline{DP}_w$ = 648 (fraction VIIB); (d) 10.3% Arg, $\overline{DP}_w$ = 236 (fraction VIIE).

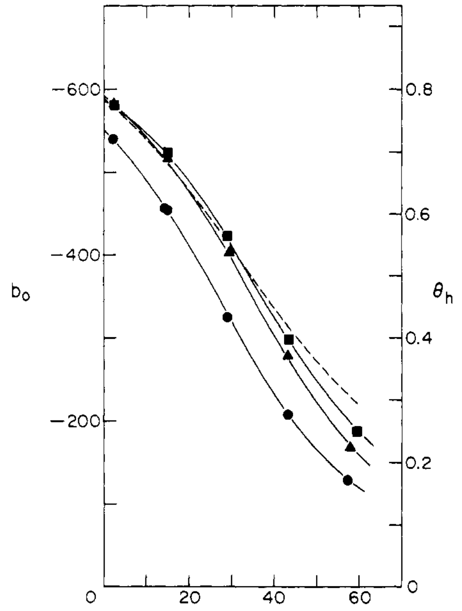

Figure 3. Thermally induced helix-coil transition for copolymer VIIIC (16.0% Arg, $\overline{DP}_w$ = 650): (●) in water; (▲) in 0.1 N KCl; (■) in 0.5 N KCl. The melting curve of PHBG ($\overline{DP}_w$ = 650), interpolated from the data of paper 2,[15] is shown as a dotted line for reference.

as the concentration of added salt was increased from 0 to 0.5 N KCl. It is apparent from these data that the conformation of this copolymer of high arginine content is influenced by long-range electrostatic interactions between the charged arginine side chains at low ionic strength. However, the small difference between the melting curves in 0.1 N KCl and 0.5 N KCl indicates that the long-range electrostatic repulsions are completely shielded in a solution of 0.5 N KCl and that fraction VIIIC in 0.5 N KCl is suitable for use in the analysis to obtain σ and s. For the remaining four fractions analyzed, all of which contain less than 10.5% arginine, no differences could be detected between the melting curves obtained in water at neutral pH and those obtained in 0.2 N KCl solutions. This indicates that, for these copolymers of low arginine content, the charged arginine side chains are sufficiently separated from one another so that the helix-coil transition is not influenced by electrostatic repulsions. All of the transition curves were found to be completely reversible, reproducible, and independent of concentration [in the range from 0.1 to 0.3% (w/v)]. The above characteristics of the helix-coil transition show that the transition curves obtained for the four fractions of low arginine content are also suitable for estimating σ and s for poly(L-arginine) in aqueous solution at neutral pH. These melting curves are shown in Figure 4.

The thermally induced melting curves demonstrate the following things. The helix content is a function of chain length and temperature. However, at low temperature, it is essentially independent of composition, indicating that arginine has a conformational preference similar to that of HBG (see the dotted lines in Figures 3 and 4). The error symbols shown in Figure 4 arise from errors in concentration (±3%), from the possible error in the choice of b_0 for the full helix and coil (±3%), and from errors in the slope of the Moffitt-Yang plot (±300/b_0%).

III. Discussion

A. Helix-Coil Parameters for Poly(L-arginine). The melting curves described in section II were analyzed according to the LAPS (Lifson-Allegra-Poland-Scheraga) hierachy of approximations to obtain the Zimm-Bragg parameters σ and s for poly(L-arginine) in aqueous solution. This procedure has been discussed extensively in previous papers in this series.[3,15,29] The approximations, corresponding to the theories of Lifson[30] and Allegra,[31] were both used to fit the data and checked with the exact method of Lehman and McTague[32] in representative cases.[33]

The results ($\theta_{h,theor}$) of these calculations are shown in Table III along with some original experimental data ($\theta_{h,exptl}$) for comparison. There are negligible differences between the values of $\theta_{h,theor}$ obtained by using the four melting curves of fractions VB, VDA, VIIB, and VIIE (Figure 4) and by using five melting curves, those of these same four fractions and that of fraction VIIIC in 0.5 N KCl (Figure 3). The theoretical helix content of fraction VIIIC was also calculated by using the data from the four other melting curves and was found to agree well with the experimental data obtained in 0.5 N KCl. This demonstrates that 0.5 N KCl completely shields the long-range electrostatic interactions between the charged arginyl residues of copolymer fraction VIIIC and that this fraction may be included in the calculation of σ and s for poly(L-arginine). Hence, all five melting curves are used in the following analysis. Both the first-order (Lifson) and the second-order (Allegra) approximations give results which agree with those obtained from the exact (Lehman-McTague) method. The higher order Allegra approximation will be used in all subsequent discussion of the arginine parameters.

The approximate theories used above for arginine appear to hold because the values of s for HBG and Arg are both close to unity over the range of the study. As was shown in paper I,[5] this behavior causes the approximations to converge very rapidly to the exact results. In fact, as we have seen, even the first-order theory is adequate for fitting the data.

The copolymer melting data were analyzed as described in paper II.[15] Treating σ as independent of temperature, the best value of σ was obtained by application of the "goodness of fit" criterion, expressed in terms of the parameter τ defined in paper II.[15] The best fit for all copolymer melting data was obtained by minimizing τ. From Figure 5, it can be seen that τ becomes insensitive to σ at approximately $\sigma = 1 \times 10^{-5}$. The value of σ obtained with the Lifson theory was similar (σ = 6 $\times 10^{-5}$). In this series, the same phenomenon, that τ becomes independent of σ at small values of σ, has been observed for glycine,[29] serine,[17,34] valine,[35] glutamic acid,[10] lysine,[3] and

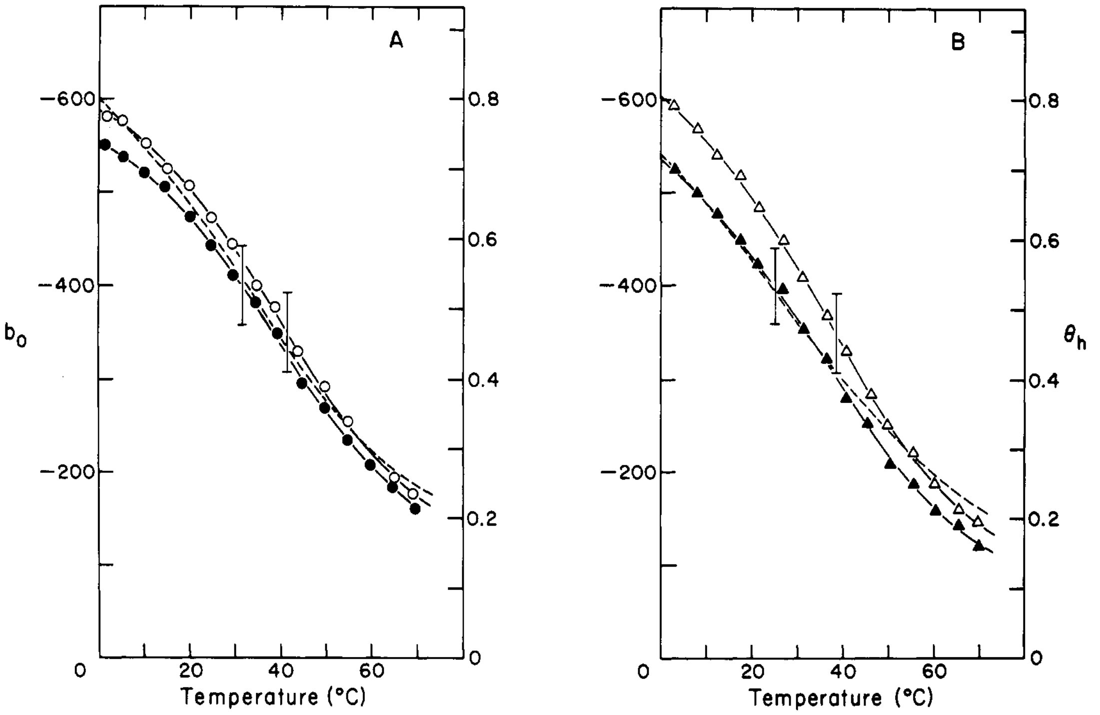

Figure 4. Temperature dependence of b_0 for poly(HBG,Arg) copolymers in water: (A) (○) 4.3% Arg, $\overline{DP}_w$ = 1036 (fraction VB), (●) 4.2% Arg, $\overline{DP}_w$ = 367 (fraction VDA); (B) (△) 10.4% Arg, $\overline{DP}_w$ = 648 (fraction VIIB), (▲) 10.3% Arg, $\overline{DP}_w$ = 236 (fraction VIIE). The melting curves of PHBG ($\overline{DP}_w$ = 1036 and 236), interpolated from the data of paper II,[15] are shown as dotted lines for reference in (A) and (B), respectively. The points are the experimental ones, and the lines represent the smoothed experimental curves. The size of the error symbols reflects the experimental error in θ_h arising from errors in the determination of concentration and in the slope of the Moffitt–Yang plot.

Table III

Comparison of the Values of θ_h Calculated with Approximate and Exact Theories for Finite Chains[a]

L-Arg content, mol %	$\overline{DP}_w$	Temp, °C	$(\theta_h)_{exptl}$	$(\theta_h)_{theor}$ Lifson[b]	Allegra[c]	Lifson[d]	Allegra[e]	Lehman–McTague[e]
4.3	1036	0	0.793	0.802	0.802	0.801	0.801	0.801
		30	0.581	0.567	0.567	0.563	0.563	0.563
		60	0.295	0.283	0.283	0.286	0.286	0.286
10.3	236	0	0.722	0.723	0.723	0.721	0.721	0.720
		30	0.492	0.503	0.503	0.495	0.496	0.493
		60	0.219	0.226	0.226	0.232	0.232	0.232
16.0	650	0	0.783			0.787	0.788	0.787
		30	0.550			0.563	0.562	0.562
		60	0.247			0.236	0.237	0.236

[a] The parameters used for hydroxybutylglutamine were taken from Table II of paper II.[15] [b] The parameters used for L-Arg were obtained by fitting the experimental data of four copolymer fractions VB, VDA, VIIB, and VIIE with the Lifson theory, as shown in Table IV. [c] The parameters used for L-Arg were obtained by fitting the experimental data of four copolymer fractions VB, VDA, VIIB, and VIIE with the Allegra theory, as shown in Table IV. [d] The parameters used for L-Arg were obtained by fitting the experimental data of five copolymer fractions VB, VDA, VIIB, VIIE, and VIIIC with the Lifson theory, as shown in Table IV. [e] The parameters used for L-Arg were obtained by fitting the experimental data of five copolymer fractions VB, VDA, VIIB, VIIE, and VIIIC with the Allegra theory, as shown in Table IV.

asparagine.[36] This behavior arises when it becomes very improbable for the guest residue to nucleate a helical segment in a pure-guest region of the random copolymer. Such nucleation becomes more improbable as the value of $\alpha\sigma_A s_A$ of the guest amino acid becomes small compared to the value of $\sigma_B s_B$ of the host amino acid, where α is the (generally small) relative composition of the guest residue (see Appendix B of paper I of this series[5]). In copolymers in which HBG, which has a large value of s and σ, is the host,[3,10,17,29,34–36] or in copolymers in which a helix breaker, which has a small value of s, is the guest residue,[17,29,34,36] a minimum in the τ vs. σ curve cannot be obtained. In copolymers with arginine as the guest residue, the independence of τ on σ (at values of $\sigma < 1 \times 10^{-5}$) occurs because the host residue (HBG) has a large value of s and σ, the guest residue has a small value of σ ($<1 \times 10^{-5}$), and the composition of the guest residue is low. The values of $s(T)$ obtained from both the Lifson and the Allegra theories are listed in Table IV. Figure 6 shows the temperature dependence of s with the estimated error in s. The error symbols in Figure 6 are the standard deviations in s at a given temperature, calculated from the best values of s determined by fitting each fraction individually at that particular temperature using a fixed value of σ ($=1 \times 10^{-5}$).

The melting curves computed with the best-fit Allegra values for σ and s are shown in Figure 7 along with the experimental points. The error symbols on the computed melting curves arise from errors in composition (±6.6%) and molecular weight (±5%). The agreement between the calcu-

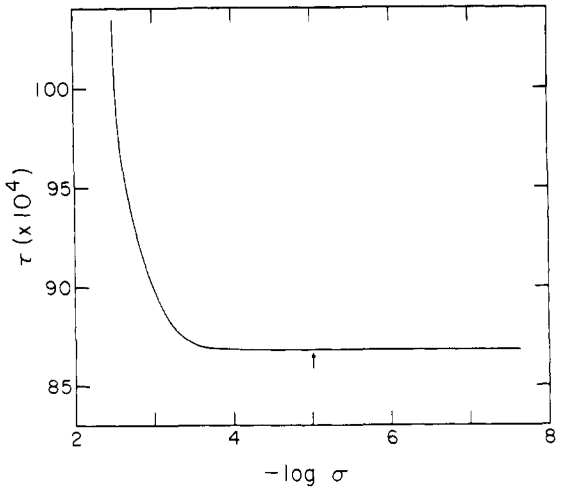

Figure 5. Determination of the best temperature-independent value of σ as the one which corresponds to the lowest value of τ for the arginine copolymers using the Allegra theory. The arrow indicates the location of a very shallow minimum.

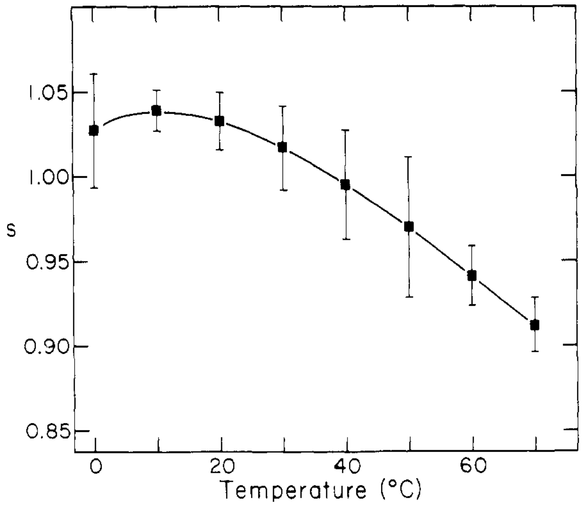

Figure 6. Temperature dependence of s for poly(L-arginine) in aqueous solution at neutral pH for $\sigma = 1 \times 10^{-5}$. The error symbols are described in section IIIA. The solid line is drawn to pass through all the points.

Table IV
Values of the Zimm–Bragg Parameters s for Poly(L-arginine) in Water or 0.2 N KCl[a] at Neutral pH

Temp, °C	s Lifson[b]	Allegra[b]	Lifson[c]	Allegra[d]
0	1.031	1.031	1.028	1.026
10	1.048	1.049	1.039	1.039
20	1.042	1.043	1.034	1.033
30	1.028	1.028	1.018	1.017
40	1.002	1.002	0.993	0.994
50	0.965	0.965	0.967	0.968
60	0.929	0.930	0.938	0.940
70	0.901	0.903	0.908	0.912

[a] For fraction VIIIC the data in 0.5 N KCl are used. [b] Calculated with $\sigma = 1 \times 10^{-6}$ and using the four melting curves of fractions VB, VDA, VIIB, and VIIE. [c] Calculated with $\sigma = 6 \times 10^{-5}$ and using the five melting curves of fractions VB, VDA, VIIB, VIIE, and VIIIC. [d] Calculated with $\sigma = 1 \times 10^{-5}$ and using the five melting curves of fractions VB, VDA, VIIB, VIIE, and VIIIC. The various values of σ arise because the use of different fractions in the calculations leads to slightly different location of the minimum in analogues of Figure 5.

lated and experimental values of θ_h is reasonable good.

The thermodynamic quantities $\Delta G°$ (the free energy), $\Delta H°$ (the enthalpy), and $\Delta S°$ (the entropy) for the conversion of a coil residue of L-arginine to a helical one at the end of a long helical sequence can be obtained from the values of s and its temperature dependence. Figure 8 shows a plot of $\Delta G°$ ($= -RT \ln s$) vs. temperature with error symbols determined from the standard deviations in s. $\Delta H°$ was calculated from the slope of the $\ln s$ vs. $1/T$ curve using the van't Hoff equation $[-dR \ln s/d(1/T) = \Delta H°]$ and $\Delta S°$ was obtained from the relation $\Delta G° = \Delta H° - T\Delta S°$. These thermodynamic parameters are listed in Table V. The $\Delta G°$ vs. temperature curve, as well as the $-R \ln s$ vs. $1/T$ curve, has a minimum in the temperature range 5–20 °C. In this region where the slope is very small, any small variation in the value of $\Delta G°$ (or s) has a large effect on the computed value of $\Delta H°$. Therefore, the values of $\Delta H°_{20}$ and $\Delta S°_{20}$ presented in Table V should be considered only as rough estimates of these quantities.

B. Comparison with Previous Results. The intrinsic helical stability of an amino acid residue has been shown to correlate well with the probability that it will be found in a helical region in proteins.[37–39] Since the guanidino group of

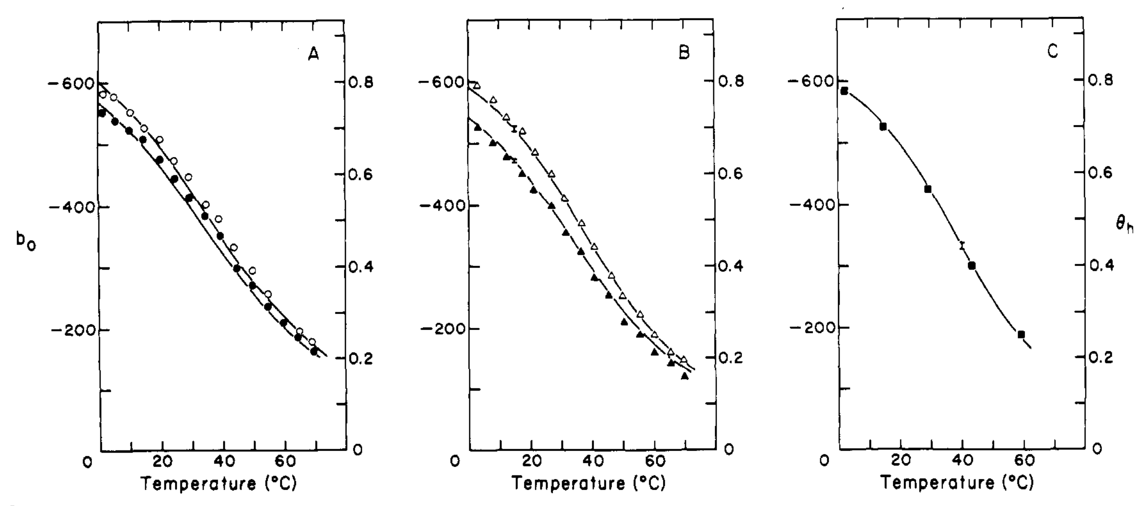

Figure 7. Comparison of the calculated melting curves, obtained with the parameters of the Allegra theory (with $\sigma = 1 \times 10^{-5}$) for L-arginine, with the experimental points: (A) (○) 4.3% Arg, $\overline{DP}_w = 1036$ (fraction VB) in water, (●) 4.2% Arg, $\overline{DP}_w = 367$ (fraction VDA) in water; (B) (△) 10.4% Arg, $\overline{DP}_w = 648$ (fraction VIIB) in water, (▲) 10.3% Arg, $\overline{DP}_w = 236$ (fraction VIIE) in water; (C) (■) 16.0% Arg, $\overline{DP}_w = 650$ (fraction VIIIC) in 0.5 N KCl. The error symbols indicate errors in the calculated values of θ_h arising from errors in composition and chain length.

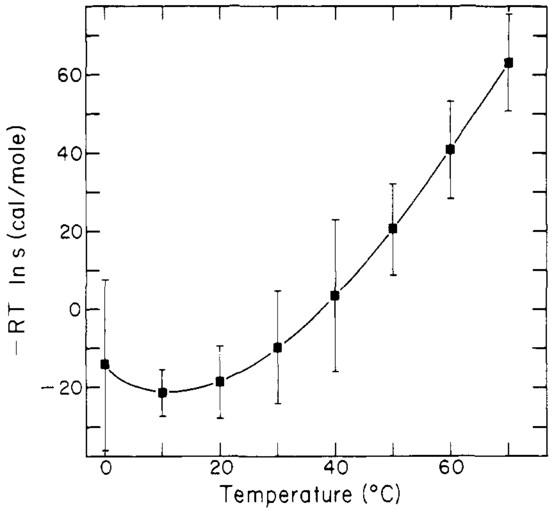

Figure 8. A plot of $-RT \ln s$ (i.e., $\Delta G°$) vs. T for poly(L-arginine) in water at neutral pH. The solid line has been drawn through the points obtained from the Allegra analysis (with $\sigma = 1 \times 10^{-5}$). The error symbols were calculated from the standard deviations in s.

Table V
Thermodynamic Parameters for L-Arginine[a]

$\Delta G°_{20}$, cal/mol	-19 ± 9
$\Delta H°_{20}$, cal/mol	-163 ± 69
$\Delta S°_{20}$, eu	-0.5 ± 0.2
σ	1×10^{-5}

[a] Calculated as described in text.

the arginine residue has a high value of pK_a (>12) in proteins,[40] the values of s for the charged arginine residue determined in this paper are expected to agree with the behavior anticipated from an examination of proteins. The theory used here[5] indicates that an arginine residue at 25 °C (where s = 1.03) has almost equal probabilities of being in the helix and coil states. In conformational analyses of proteins, arginine has been classified as helix indifferent,[39,41,42] neutral,[43] weakest helix making,[44,45] and having weakly positive helix-forming power[46] or information.[47] The results from both the random copolymers and proteins thus agree, indicating that the conformational state of arginine is determined by short-range, nearest-neighbor interactions in both types of polymeric systems. On the other hand, longer range interactions in proteins have been examined for all charged amino acids;[37] such interactions arise from the presence of an oppositely charged residue four residues away from a given residue along the chain and enhance its probability for being helical.[37,48-51] The relatively low values of s of glutamic acid and lysine, compared with their conformational preferences in proteins, were interpreted in terms of such long-range interactions. In contrast, such long-range interactions [at least at *low* Arg content in poly(HBG,Arg)] do not influence the value of s of Arg. The corresponding behavior of Arg in proteins (i.e., its characterization as helix indifferent) probably reflects an average behavior arising from the facts that (i) many of the Arg residues in proteins are near other charged residues (and the mutual electrostatic interactions are probably many body rather than pairwise effects), (ii) Arg residues are approximately equally distributed over the inside and outside of protein molecules,[52] and (iii) Arg is often in specific catalytic sites (generally the charge-binding sites for enzymes acting on anionic substrates[53,54]). Thus, we have agreement for the behavior of Arg in the random copolymers studied here (where only nearest-neighbor interactions are involved) and in proteins [where long-range interactions are also involved, but probably cancel out to a large extent because of effects i to iii].

IV. Conclusion

Water-soluble random copolymers of L-arginine and N^5-(4-hydroxybutyl)-L-glutamine were synthesized and characterized. From an analysis of the thermally induced helix–coil transition of these copolymers, the Zimm–Bragg parameters σ and s for poly(L-arginine) were determined. The values of s show that L-arginine is a weak helix former at low temperature and a weak helix breaker at high temperature. It has been shown that, in order to measure the intrinsic conformational preference of charged residues, the arginines must be sufficiently isolated from one another so that the long-range electrostatic repulsions do not influence the conformation. The host–guest technique is adequate for this purpose.

Acknowledgment. We are indebted to Mr. T. Thannhauser for performing the nitrogen and amino acid analyses and to Dr. B. Zweifel, Dr. F. R. Maxfield, and Mr. R. R. Matheson, Jr., for helpful discussions.

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Helix–Coil Stability Constants for the Naturally Occurring Amino Acids in Water. 16. Aspartic Acid Parameters from Random Poly(hydroxybutylglutamine-*co*-L-aspartic acid)[1]

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Received July 25, 1977

ABSTRACT: The synthesis and characterization of water-soluble random copolymers containing L-aspartic acid with N^5-(4-hydroxybutyl)-L-glutamine, and the thermally induced helix–coil transitions of these copolymers in water and in 0.1 N KCl, are described. The incorporation of L-aspartic acid was found to decrease the helix content of the polymer at both high and low pH, in water and also in 0.1 N KCl. The Zimm–Bragg parameters σ and s for the helix–coil transition in poly(L-aspartic acid) in water and in 0.1 N KCl were deduced from an analysis of the melting curves of the copolymers in the manner described in earlier papers. Corrections were made for the presence of a small amount of racemized aspartic acid, using data from random copolymers containing D-aspartic acid as the guest residue. The computed values of s indicate that L-aspartic acid destabilizes helical sequences at all temperatures in the range of 0–70 °C. Titrations of the copolymers and of N-acetyl-N'-methyl-L-aspartic acid amide in 0.1 N KCl are described.

There have been several investigations of the conformation of poly(L-aspartic acid) in aqueous solutions.[3–9] Little information, however, is available for the conformational preference of the L-aspartic acid residue, because of difficulties in the synthesis and quantitative analysis of polymers containing L-aspartic acid. These investigations suggest that neutral poly(L-aspartic acid) forms a right-handed α helix in water with a less organized helical structure than its homologue poly(L-glutamic acid)[6,7] and does not display a detectable cooperative helix–coil transition throughout its range of ionization.[8]

The unusually large tendency for aspartic acid to undergo racemization and transamidation introduces difficulties in the synthesis of poly(L-aspartic acid).[3–5] Difficulties in the analysis of the properties of this homopolymer arise from the presence of charged residues which complicate the interpretation of the results.[7,8] To eliminate these difficulties and to gain insight into the conformational behavior of L-aspartic acid residues in proteins, water-soluble random copolymers of L-aspartic acid and a nonionizable "host", N^5-(4-hydroxybutyl)-L-glutamine (HBG), have been synthesized. By application of the host–guest technique described and applied in previous papers of this series, the most recent one being that of Konishi et al.,[10] the Zimm–Bragg helix–coil stability constants,[11] σ and s, of L-aspartic acid were determined in both the charged and uncharged states. Random copolymers of N^5-(4-hydroxybutyl)-L-glutamine with D-aspartic acid were also synthesized and their helix–coil stability constants were determined in order to correct for the contribution from the racemized residues to the helix–coil stability constants of L-aspartic acid.

The results presented here indicate that L-aspartic acid is a pronounced helix breaker in water over the temperature range 0–70 °C and confirm the earlier qualitative work that poly(L-aspartic acid) has a low helix content in aqueous solution. They also demonstrate that there is a difference between the helix-forming abilities of charged and uncharged residues of aspartic acid, similar to the cases of L-glutamic acid and L-lysine in papers IX and XI of this series;[12,13] i.e., charged L-aspartic acid is intrinsically a stronger helix breaker than is the uncharged form.

The synthesis of water-soluble random copolymers of L- and D-aspartic acids, respectively, with N^5-(4-hydroxybutyl)-L-glutamine, and their fractionation, are described in section I. The characterization of these copolymers and their melting and titration behavior are presented in section II. Finally, the data are analyzed to determine the helix–coil stability parameters of L-aspartic acid in their uncharged and charged forms in water and in 0.1 N KCl in section III. These results are then compared with previous results for polypeptides and proteins.

I. Experimental Section. Preparation and Characterization of the Copolymers

The copolymers were prepared from the N-carboxyanhydrides of γ-benzyl L-glutamate and β-*tert*-butyl L-aspartate (or D-aspartate)